

**PETITION FOR A PERMANENT EXEMPTION FROM THE  
REQUIREMENT OF A TOLERANCE FOR**

Modified Cry3A Protein and the Genetic Material Necessary for its Production  
in All Corn

**SUBMITTED BY**

Syngenta Seeds, Inc.  
P.O. Box 12257  
3054 Cornwallis Road  
Research Triangle Park, North Carolina 27709-2257

**DATE**

April 28, 2004

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Federal Register Notice of Filing and Computer Disk



Syngenta Seeds, Inc. – Field Crops - NAFTA  
P. O. Box 12257  
3054 Cornwallis Road  
Research Triangle Park, North Carolina 27709-2257

April 28, 2004

Biopesticide and Pollution Prevention Division  
Environmental Protection Agency  
Washington, DC 20460

To Whom It May Concern:

Syngenta Seeds, Inc. – Field Crops – NAFTA submits this petition for a permanent exemption from the requirement of a tolerance pursuant to section 408(d)(1) of the Federal Food, Drug, and Cosmetic Act with respect to the pesticide chemical (plant incorporated protectant) modified Cry3A protein and the genetic material necessary for its production in all corn. Concurrent with the submission of the petition, Syngenta Seeds is submitting an application for FIFRA Section 3 Registration of this plant-incorporated protectant as expressed in Event MIR604 corn plants.

Attached hereto, in duplicate and constituting a part of this petition, are the following:

- A. The name, chemical identity, and composition of the pesticide chemical.
- B. The amount, frequency, and time of application of the pesticide chemical.
- C. Full reports of investigations made with respect to the safety of the pesticide chemical.
- D. The results of tests on the amount of residue remaining, including a description of the analytical method used.
- E. Practicable methods for removing residue that exceeds any proposed tolerance.
- F. Proposed tolerances for the pesticide chemical if tolerances are proposed.
- G. Reasonable grounds in support of the petition.

Pursuant to the recently enacted pesticide registration service fee schedule<sup>1</sup>, tolerance petition fees are no longer applicable. Accordingly, Syngenta is not enclosing payment of a tolerance fee in connection with this Petition, as we expect to be invoiced for the pesticide registration service fee associated with the regulatory action we have requested. Please contact me if you have any questions.

Sincerely,

A handwritten signature in cursive script, reading "Demetra Vlachos".

Demetra Vlachos  
Senior Manager, Regulatory Affairs

Tel. 919-541-8572

Fax. 919-541-8535

E-mail: demetra.vlachos@syngenta.com

Enclosure: Petition for Tolerance Exemption (in duplicate)

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<sup>1</sup> As described in the *Federal Register*, vol. 69 (52), pages 12772 – 12780; March 17, 2004.

## SECTION A

### NAME, CHEMICAL IDENTITY, AND COMPOSITION OF THE PESTICIDE

A permanent exemption from the requirement for a tolerance is proposed for the plant-incorporated protectant ‘modified Cry3A protein and the genetic material necessary for its production in all corn.’ This petition is being submitted concurrently with an application for FIFRA Section 3 registration of this active ingredient in Event MIR604 corn plants. Information about the identity and composition of the active ingredient can be found in the following data volumes submitted previously to EPA in support of an Experimental Use Permit application (67979-EUP-U) for Event MIR604 corn plants, which contain the modified Cry3A plant-incorporated protectant:

<b>Volume Title</b>	<b>MRID Number</b>
Characterization and Safety of Modified Cry3A <i>Bacillus thuringiensis</i> Protein and Maize (Corn) Plants Derived from Event MIR604	46155601
Molecular Characterization of Event MIR604 Maize (Corn) Expressing a Modified Cry3A <i>Bacillus</i> <i>thuringiensis</i> Protein	46155602

## **SECTION B**

### **AMOUNT, FREQUENCY, AND TIME OF APPLICATION**

The active ingredient is a plant-incorporated protectant that is produced within transgenic corn plants. A copy of the proposed registration label for modified Cry3A protein and the genetic material necessary for its production in Event MIR604 corn is provided herein.

## **Event MIR604 Rootworm-Protected Corn**

### **Plant-Incorporated Protectant: Modified Cry3A Protein for Corn Rootworm Control**

This product is effective in controlling or suppressing damage caused by larvae of some corn rootworm species feeding on corn roots.

#### **Active Ingredient:**

Modified Cry3A protein and the genetic material necessary for its production (*via* elements of pZM26) in Event MIR604 corn..... $\leq 0.0005\%$ \*

#### **Other Ingredients:**

Phosphomannose isomerase, a marker protein, and the genetic material necessary for its production (*via* elements of pZM26) in Event MIR604 corn..... $\leq 0.0001\%$ \*

\*Percentage in seed on a dry weight basis.

**Keep Out of Reach of Children**

### **CAUTION**

EPA Registration No. 67979-

EPA Establishment No. 66736-NC-001

Syngenta Seeds, Inc. - Field Crops – NAFTA

P.O. Box 12257

3054 Cornwallis Rd.

Research Triangle Park, NC 27709

## **DIRECTIONS FOR USE**

It is a violation of Federal law to use this product in a manner inconsistent with its labelling.

The following information regarding commercial planting of Event MIR604 hybrid field corn must be included in the Grower Guide for Event MIR604 Rootworm-Protected Corn.

### **Insect Resistance Management**

Growers of Event MIR604 Rootworm-Protected Corn must adhere to the following refuge requirements. Growers must plant a structured refuge of at least 20% non-Event MIR604 corn. Other transgenic corn products for rootworm control are not permitted as refuge corn.

Refuge planting options include adjacent blocks or in-field strips. If blocks are used they must be adjacent to the Event MIR604 corn field. If strips within a corn field are used, then at least 6, and preferably 12, consecutive rows should be planted with refuge corn.

The refuge and Event MIR604 corn acres should be managed under comparable agronomic regimes. If irrigation is used, both the refuge and the Event MIR604 corn acres should be irrigated. In regions where corn is cropped continuously, both the refuge and the Event MIR604 corn acres should be planted in a continuous cropping regime. The refuge may be placed only on first-year corn acres if the Event MIR604 corn is planted on first-year corn acres.

Growers have the option of applying conventional insecticides to the refuge corn for control of corn rootworm larvae. Growers are not permitted to apply agents for control of adult corn rootworms (corn rootworm beetles) to the refuge, as this would render the refuge less effective. If growers opt to treat for other insects present in the refuge while adult corn rootworms are present, then the Event MIR604 corn acres must be treated in a like manner.

These refuge requirements do not apply to operations related to the propagation of Event MIR604 inbred seed, to the production of Event MIR604 hybrid seed for future commercial sale, or to non-commercial, small-scale plantings of MIR604 field corn hybrids solely for research purposes.

### **Corn Insects Controlled or Suppressed**

Corn has been genetically transformed to produce a modified Cry3A protein for control or suppression of the following coleopteran insects:

Western corn rootworm (*Diabrotica virgifera virgifera*)

Northern corn rootworm (*Diabrotica longicornis barberi*)

Mexican corn rootworm (*Diabrotica virgifera zea*)

## SECTION C

### FULL REPORTS ON INVESTIGATIONS MADE WITH RESPECT TO THE SAFETY OF THE PESTICIDAL PRODUCT

The following data volumes describe the safety of the modified Cry3A protein and the genetic material necessary for its production in corn.

<u>Study Title</u>	<u>MRID Number</u>
Characterization and Safety of Modified Cry3A <i>Bacillus thuringiensis</i> Protein and Maize (Corn) Plants Derived from Event MIR604	46155601
Molecular Characterization of Event MIR604 Maize (Corn) Expressing a Modified Cry3A <i>Bacillus</i> <i>thuringiensis</i> Protein	46155602
Characterization of Modified Cry3A Protein Produced in Event MIR604-Derived Maize (Corn) and Comparison with Modified Cry3A Protein Expressed in Recombinant <i>Escherichia coli</i>	46155603
Characterization of Modified Cry3A Test Substance MCRY3A-0102 and Certificate of Analysis	46155605
Further Characterization of Modified Cry3A Test Substance MCRY3A-0102	46155606
<i>In vitro</i> Digestibility of Modified Cry3A Protein (MCRY3A-0102 and IAPMIR604-0103) Under Simulated Gastric Conditions	46155607
Effect of Temperature on the Stability of Modified Cry3A Protein (MCRY3A-0102)	46155608
Acute Oral Toxicity Study of Modified Cry3A Protein (MCRY3A-0102) in the Mouse	46155610
Modified Cry3A Protein as Expressed in Transgenic Maize Event MIR604: Assessment of Amino Acid Homology with Known Toxins	46155611
Modified Cry3A Protein as Expressed in Transgenic Maize Event MIR604: Assessment of Amino Acid Homology with Known Allergens	46155612



## SECTION D

### RESULTS OF TESTS ON THE AMOUNT OF RESIDUE REMAINING, INCLUDING A DESCRIPTION OF THE ANALYTICAL METHOD USED

Data describing the residues and the residue profile for the modified Cry3A protein are contained in the following volumes. A detailed description of the analytical method used to quantify mCry3A is included in the first volume listed:

<b>Study Title</b>	<b>MRID Number</b>
Quantification of Modified Cry3A and PMI Proteins in Transgenic Maize (Corn) Tissues, Whole Plants, and Silage Derived from Transformation Event MIR604	46155604
Analysis for the Presence of Modified Cry3A Protein in Wet and Dry Milled Fractions, Corn Oil and Corn Chips from Corn (Maize) Event MIR604	46155609

## **SECTION E**

### **PRACTICAL METHODS FOR REMOVING RESIDUE THAT EXCEEDS ANY PROPOSED TOLERANCE**

Given that an exemption from the requirement of a tolerance is being proposed, this section is not applicable.

## **SECTION F**

### **PROPOSED TOLERANCE FOR THE PESTICIDE CHEMICAL IF TOLERANCES ARE PROPOSED**

Given that an exemption from the requirement of a tolerance is being proposed, this section is not applicable.

## **SECTION G**

### **REASONABLE GROUNDS IN SUPPORT OF THIS PETITION**

Reasonable grounds to support this petition for a permanent exemption from the requirement of a tolerance for modified Cry3A protein and the genetic material necessary for its production in all corn are presented in the summaries found in the Notice of Filing document that follows.

## **PROPOSED NOTICE OF FILING**

**FILE NAME: biopetit.wpt (7/1/2003) (xml)**

**ATTENTION:**

**All commodity terms must comply with the Food and Feed Commodity Vocabulary database (<http://www.epa.gov/pesticides/foodfeed/>).**

**All text in blue font (instructions for preparing the document), should be removed prior to sending the document to the Federal Register Staff. Instructional text and prompts in green font should also be removed.**

**EPA BIOPESTICIDES AND POLLUTION PREVENTION DIVISION COMPANY  
NOTICE OF FILING TEMPLATE FOR PESTICIDE PETITIONS PUBLISHED  
IN THE FEDERAL REGISTER  
(7/1/2003)**

**EPA Biopesticides and Pollution Prevention Division contact: [insert name and telephone number with area code]**

**TEMPLATE:**

**[Insert company name]**

**[Insert petition number]**

EPA has received a pesticide petition [PF- ] from [Syngenta Seeds, Inc.] , [P.O. Box 12257, 3054 Cornwallis Road, Research Triangle Park, NC 27709-2257], proposing pursuant to section 408(d) of the Federal Food, Drug, and Cosmetic Act (FFDCA), 21 U.S.C. 346a(d), to amend 40 CFR part 180 to establish an exemption from the requirement of a tolerance for the plant-pesticide [modified Cry3A protein and the genetic material necessary for its production] in or on [all corn].

Pursuant to section 408(d)(2)(A)(i) of the FFDCA, as amended, [Syngenta Seeds, Inc.] has submitted the following summary of information, data, and arguments in support of their pesticide petition. This summary was prepared by [Syngenta Seeds, Inc.] and EPA has not fully evaluated the merits of the pesticide petition. The summary may have been edited by EPA if the terminology used was unclear, the summary contained extraneous material, or the summary unintentionally made the reader conclude that the findings reflected EPA's position and not the position of the petitioner.

**I. [Syngenta Seeds, Inc] Petition Summary**

Syngenta Seeds

[PF-]

*A. Product name and Proposed Use Practices*

[A modified Cry3A insect control protein and the genetic material necessary for its production in all corn is proposed for use as a plant-incorporated protectant active ingredient. Production of the modified Cry3A protein within corn plants confers resistance to damage caused by the western corn rootworm and northern corn rootworm, which are major corn pests in the US. A permanent exemption from tolerances is being requested in conjunction with an application for commercial FIFRA Section 3 registration of the active ingredient for use in corn. ]

*B. Product Identity/Chemistry*

1. *Identity of the pesticide and corresponding residues.* [A modified Cry3A insect control protein is produced in transgenic corn plants derived from transformation Event MIR604. A *cry3A* gene from *Bacillus thuringiensis* subsp. *tenebrionis* was recreated synthetically to optimize for expression in corn. Additional changes in this corn-optimized gene were made, such that the encoded modified Cry3A protein (mCry3A) has enhanced activity against larvae of the western corn rootworm (*Diabrotica virgifera virgifera*) and northern corn rootworm (*D. longicornis barberi*). Event MIR604-derived corn plants express the synthetic modified *cry3A* gene, introduced *via* transformation vector pZM26, and display resistance to these pests. The native Cry3A protein of *B. thuringiensis* subsp. *tenebrionis* is a *ca.* 73 kDa polypeptide of 644 amino acids. By comparison, the mCry3A protein expressed in Event MIR604 corn is a *ca.* 67 kDa polypeptide of 598 amino acids. Its amino acid sequence corresponds to that of the native Cry3A protein, except that (1) its N-terminus corresponds to methionine-48 of the native protein and (2) a cathepsin G protease recognition site has been introduced into the protein, conferring markedly enhanced commercially exploitable activity toward western and northern corn rootworms. Residues of the mCry3A protein, and/or breakdown products thereof, are present in corn grain and other tissues of Event MIR604-derived plants. ]

2. *Magnitude of residue at the time of harvest and method used to determine the residue.* [A determination of the magnitude of residue at harvest is not required for residues exempt from tolerances. However, the petitioner has provided data on the quantity of mCry3A protein measured in various plant parts. Average mCry3A levels in grain from Event MIR604-derived hybrid field corn plants were less than one part per million (ppm) on a dry- or fresh-weight basis, as measured by ELISA. Average mCry3A levels measured in chopped whole Event MIR604-derived hybrid corn plants were less than or equal to *ca.* 20 ppm on a dry-weight basis and less than or equal to *ca.* 8 ppm on a fresh-weight basis. ]

3. *A statement of why an analytical method for detecting and measuring the levels of the pesticide residue are not needed.* [An analytical method is not required

because this petition requests an exemption from tolerances. However, the petitioner has submitted an analytical method for detection of the mCry3A protein by ELISA.]

### *C. Mammalian Toxicological Profile*

[Syngenta Seeds has provided the results of a mammalian toxicology study, *in vitro* digestibility study, heat stability study and bioinformatics evaluations conducted on the mCry3A protein. These studies, summarized herein, demonstrate the lack of toxicity of the mCry3A protein following acute oral high-dose exposure to mice, rapid degradation of mCry3A upon exposure to simulated mammalian gastric fluid, instability of the mCry3A protein upon heating, and the lack of significant amino acid sequence homology of the mCry3A protein to proteins known to be mammalian toxins or human allergens.

When proteins are toxic, they are known to act *via* acute mechanisms and at very low doses [Sjogblad, R.D., J.T. McClintock and R. Engler (1992) Toxicological considerations for protein components of biological pesticide products. *Regulatory Toxicol. Pharmacol.* 15: 3-9]. Therefore, when a protein demonstrates no acute oral toxicity in high-dose testing using a standard laboratory mammalian test species, this supports the determination that the protein will be non-toxic to humans and other mammals, and will not present a hazard under any realistic exposure scenario, including long-term exposures.

Because it is not feasible to extract sufficient mCry3A protein from transformed plants for high-dose toxicology studies, mCry3A protein was produced in recombinant *E. coli* by over-expressing the same modified *cry3A* gene that was introduced into Event MIR604 corn plants. Following purification from *E. coli*, dialysis and lyophilization, the resulting sample, designated test substance MCRY3A-0102, was estimated by ELISA to contain *ca.* 90.3% mCry3A protein by weight. Side-by-side comparisons of mCry3A in test substance MCRY3A-0102 with mCry3A extracted from Event MIR604-derived corn plants indicated that mCry3A from both sources is biologically active against the same target pest species, has the same apparent molecular weight by SDS-PAGE, immunoreacts with the same anti-Cry3A antibody, and is not apparently glycosylated post-translation. Additionally, peptide mapping of *ca.* 60% of the mCry3A polypeptide by mass-spectral analysis confirmed the identity and intended amino sequence of mCry3A in test substance MCRY3A-0102. Nucleotide sequencing of the entire DNA insert in Event MIR604-derived plants also confirmed that the mCry3A protein produced in the plants has the exact intended amino acid sequence. These data justify the use of test substance MCRY3A-0102 in safety studies as a surrogate for mCry3A as produced in Event MIR604-derived plants.

An acute toxicity study was conducted in mice according to US EPA Test Guideline OPPTS 870.1100. Test substance MCRY3A-0102 was administered orally by gavage to 5 male and 5 female mice at a dose of 2632 mg/kg body weight, representing *ca.* 2377 mg of pure mCry3A protein/kg body weight. A negative



control group (5 males and 5 females) concurrently received the dosing vehicle alone, an aqueous suspension of 1% methylcellulose, at the same dosing volume used for the test substance mixture. No test substance-related mortalities or clinical signs of toxicity occurred during the 14-day study. One female mouse in the test group was euthanized the day following dosing due to adverse clinical signs resulting from a dosing injury (confirmed by post-mortem examination). At study termination, macroscopic and microscopic examination of all major organs of the surviving mice revealed no treatment-related abnormalities. Body weight, body weight gain and organ weights (brain, liver, kidneys and spleen) were comparable in the control and test groups. There was no evidence of toxicity. Accordingly, the LD<sub>50</sub> value for MCRY3A-0102 in male and female mice is greater than 2632 mg/kg body weight, and the LD<sub>50</sub> value for pure mCry3A protein is greater than 2377 mg/kg body weight, the single dose tested.

Extensive bioinformatics searches of public protein databases revealed that the mCry3A protein shows no significant amino acid homology to proteins known to be mammalian toxins or known or suspected to be human allergens. Additional information and testing indicate that the mCry3A protein does not have properties that would suggest it has the potential to become a food allergen. The source of native Cry3A protein (*Bacillus thuringiensis*) is not known to produce food allergens. Unlike allergenic proteins, which typically are present at 1 – 80% of the total protein in an offending food, the average mCry3A concentration measured in raw grain derived from Event MIR604 corn represents less than 0.0001% of the total protein. (This calculation is based on corn grain containing 10% total protein by weight, and assumes less than 1 ppm mCry3A in the grain.) Additionally, due to degradation *via* food processing methods, mCry3A will not likely be present in processed food products, or will be present in only trace quantities. The mCry3A protein produced in transformed corn plants is not targeted to a cellular pathway for glycosylation, and shows no evidence of post-translational glycosylation. Bioactivity of mCry3A is lost upon heating at 95°C for 30 minutes. Upon exposure to simulated mammalian gastric fluid containing pepsin, mCry3A rapidly degrades.

The native Cry3A protein has had a history of safe use as a component of spore preparations of the microbial insecticide *B. thuringiensis* subsp. *tenebrionis*, as an encapsulated component of a microbial insecticide derived from *B. thuringiensis* subsp. *san diego*, and as a plant-incorporated protectant in Bt potato.

The genetic material occurring in the subject plant-incorporated protectant active ingredient has been adequately characterized. This genetic material (*i.e.*, the nucleic acids DNA and RNA), including regulatory regions, necessary for the production of mCry3A in all corn will not present a dietary safety concern. “Regulatory regions” are the DNA sequences such as promoters, terminators, and enhancers that control the expression of the genetic material encoding the protein. Based on the ubiquitous occurrence and established safety of nucleic acids in the food supply, a tolerance exemption under the Federal Food Drug and Cosmetic Act regulations has been established for residues of nucleic acids that are part of plant-incorporated protectants [40 CFR Part 174.475; *Fed. Reg.* 66(139): 37817-37830,

July 19, 2001]. Therefore, no mammalian toxicity is anticipated from dietary exposure to the genetic material necessary for the production of mCry3A protein in all corn. ]

#### *D. Aggregate Exposure*

##### *1. Dietary exposure.*

i. *Food.* [Average mCry3A levels measured in grain from Event MIR604-derived hybrid field corn plants were less than one part per million (ppm) on a dry- or fresh-weight basis. Processed corn products or by-products used in food are unlikely to have measurable mCry3A protein, or will have only trace amounts. Oral exposure is not expected to result in adverse health effects, because of a demonstrated lack of toxicity to mammals and the rapid digestibility of the mCry3A protein. It is expected that any mCry3A protein consumed will be digested as conventional dietary protein.]

ii. *Drinking water.* [Little to no exposure *via* drinking water is anticipated. Due to the demonstrated mammalian safety profile of mCry3A, such exposure would not present a risk.]

2. *Non-dietary exposure.* [Non-dietary exposure is not anticipated, due to the proposed use pattern of the product. Exposure *via* dermal or inhalation routes is unlikely because the active ingredient is contained within plant cells. However, if exposure were to occur by non-dietary routes, no risk would be expected because the mCry3A protein is not toxic to mammals.]

#### *E. Cumulative Exposure*

[Because there is no indication of mammalian toxicity of the mCry3A protein or the genetic material necessary for its production, it is reasonable to conclude that there will be no cumulative effects for this active ingredient.]

#### *F. Safety Determination*

1. *U.S. population.* [The lack of mammalian toxicity at high levels of exposure to the mCry3A protein demonstrates the safety of the product at levels well above possible maximum exposure levels anticipated *via* consumption of all food commodities produced from corn plants that produce mCry3A. Moreover, little to no human dietary exposure to mCry3A protein is expected to occur *via* transformed corn. Due to the digestibility and lack of toxicity of the mCry3A protein, and its very low potential to become an allergen in food, dietary exposure, if it occurred, is expected to not pose any harm for the U.S. population. No special safety provisions are applicable for consumption patterns or for any population sub-groups.]

**2. Infants and children.** [Based on the mammalian safety profile of the active ingredient and the proposed use pattern, there is ample evidence to conclude a reasonable certainty of no harm to infants and children.]

*G. Effects on the Immune and Endocrine Systems*

[The active ingredient is derived from sources that are not known to exert an influence on the endocrine or immune systems.]

*H. Existing Tolerances*

[The registrant is not aware of any existing tolerances or tolerance exemptions for mCry3A protein and the genetic material necessary for its production as an active ingredient. The applicant has previously submitted a petition (File Symbol 4G6808) for temporary exemption from tolerances for the same active ingredient concurrently with an application for an Experimental Use Permit for use of the active ingredient in Event MIR604 corn. Exemptions from tolerances exist for use of the native form of Cry3A protein as a plant-incorporated protectant in Bt potato (40 CFR 180.1147) and as a component of an encapsulated *Bacillus thuringiensis* microbial insecticide (40 CFR 180.1108).]

*I. International Tolerances*

[No Codex maximum residue levels exists for the plant-incorporated protectant modified Cry3A protein and the genetic material necessary for its production in corn.]